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(54) **Title:** EYE-DROP VACCINE CONTAINING COPOLYMER POLY-YE FOR THERAPEUTIC IMMUNIZATION

(57) **Abstract:** The invention provides an eye-drop vaccine for therapeutic immunization of a mammal comprising the copolymer poly-YE, also designated poly-Glu,Tyr, for treating neuronal degeneration caused by an injury or disease, disorder or condition in the central nervous system (CNS) or peripheral nervous system (PNS), for preventing or inhibiting neuronal secondary degeneration which may otherwise follow primary injury in the CNS, for promoting nerve regeneration in the CNS or in the PNS after an injury, disease, disorder or condition or for protecting CNS and PNS cells from glutamate toxicity

WO 2004/060266 A2

EYE-DROP VACCINE CONTAINING THE COPOLYMER POLY-YE FOR THERAPEUTIC IMMUNIZATION

FIELD OF THE INVENTION

The present invention is in the field of Immunology and relates to an eye-drop vaccine comprising a random copolymer, in particular the copolymer poly-YE, as the active agent, and to methods for neuroprotection of the nervous system which comprises therapeutic immunization with said eye-drop vaccine.

BACKGROUND OF THE INVENTION

The nervous system comprises the central and the peripheral nervous system. The central nervous system (CNS) is composed of the brain and spinal cord; the peripheral nervous system (PNS) consists of all of the other neural elements, namely the nerves and ganglia outside of the brain and spinal cord.

Damage to the nervous system may result from a traumatic injury, such as penetrating trauma or blunt trauma, or a disease, disorder or condition, including but not limited to Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, diabetic neuropathy, glaucoma, senile dementia, and ischemia.

Neurodegenerative disorders are commonly associated with ongoing neuronal loss in the CNS. Following the loss of neurons caused by primary risk factors, additional ("secondary") neuronal loss is mediated by self-compounds, such as glutamate, nitric oxide or reactive oxygen species, that exceed their physiological concentrations. These compounds are implicated in various types of neurological disorders and acute CNS injuries. It is interesting to note that the destructive components common to neurodegenerative diseases have also been identified in autoimmune diseases such as multiple sclerosis; in this disease, myelin damage in the CNS is accompanied by subsequent neuronal loss

The mechanism underlying progressive loss of vision in glaucoma has been suggested as similar to that occurring in any neurodegenerative disease of the CNS (Schwartz et al., 1996). According to this proposal, it is assumed that the primary loss is initiated by a primary risk factor that promotes a cascade of events leading to secondary degeneration of cells in the vicinity of the damaged area. Several mediators and pathways have been identified in the various neurodegenerative diseases as responsible for the continuous ongoing loss of neurons, even if the primary risk factor has been alleviated.

It has been recently observed by the present inventors that under neurodegenerative conditions caused by mechanical (axotomy) or biochemical (glutamate, oxidative stress) insults, the immune system plays a critical role. Thus, it has been found that activated T cells that recognize an antigen of the nervous system (NS) promote nerve regeneration or confer neuroprotection, as described for example in PCT Publication No. WO 99/60021. More specifically, T cells reactive to MBP were shown to be neuroprotective in rat models of partially crushed optic nerve (Moalem et al., 1999) and of spinal cord injury (Hauben et al., 2000). Until recently, it had been thought that the immune system excluded immune cells from participating in nervous system repair. It was quite surprising to discover that NS-specific activated T cells could be used to promote nerve regeneration or to protect nervous system tissue from secondary degeneration which may follow damage caused by injury or disease of the CNS or PNS.

It was further observed by the present inventors that stressful conditions in the CNS harness the adaptive immune response to cope with the stress and that this response is genetically controlled. Thus, the survival rate of retinal ganglion cells in adult mice or rats after crush injury of the optic nerve or intravitreal injection of a toxic dosage of glutamate was shown to be up to two-fold higher in strains that are resistant to CNS autoimmune diseases than in susceptible strains. The difference was found to be attributable to a beneficial autoimmune T cell response that was spontaneously evoked after CNS insult in the resistant but not in susceptible strains.

Thus, the survival rate of neurons as a result of such an insult is higher when T cell response directed against self is evoked, provided that it is well-regulated. In other words, it was demonstrated that a protective autoimmune response is evoked to oppose the stressful conditions so as to protect the animal from the insult consequences. It was further observed that in animals with an impaired ability to regulate such a response, or in animals devoid of mature T cells (as a result of having undergone thymectomy at birth), the ability to cope with the stressful conditions is reduced. Consequently, the survival rate of neurons following CNS insult in these animals is significantly lower than in animals endowed with an effective mechanism for mounting protective autoimmune T cell-mediated response (Kipnis et al., 2001).

More recent studies in our laboratory have shown that autoimmune neuroprotection is the body's physiological defense mechanism awakened when CNS injury occurs (Kipnis et al., 2001; Yoles et al., 2001). We demonstrated that resistance to increased intraocular pressure (IOP) differs among strains (Bakalash et al., 2002) and that this difference is linked to the ability to harness an autoimmune response with a beneficial outcome. We further showed that in the absence of mature T cells (through neonatal thymectomy), the relative resistance to IOP elevation loses its beneficial trait, and vice versa, when splenocytes from a resistant strain are passively transferred to an MHC-matched susceptible strain, the neuroprotective effect is resumed (Bakalash et al., 2002). It was further shown by our group that passive vaccination with T cells is also effective in acute injuries such as partial optic nerve crush or spinal cord contusion (Kipnis et al., 2001; Moalem et al., 1999).

Attempting to boost such an anti-self response as a way of protecting neurons from insulting conditions has revealed that the vaccinating antigen should be derived from compounds residing in the site of the lesion. Thus, the use of the self-antigen derived from interphotoreceptor binding protein (IRBP), the most abundant peptide in the eye (Bakalash et al., 2002; Mizrahi et al., 2002), resulted in retinal ganglion cells (RGC) protection in both susceptible and resistant strains. In contrast, the use of

peptides derived from compounds residing in the myelin associated with the optic nerve led to no benefit to the retinal ganglion cells suffering from IOP elevated insult.

Trying to design a vaccination for glaucoma that will boost the immune system without risk of evoking an autoimmune disease, we chose to focus on Cop-1, an FDA-approved drug for multiple sclerosis, and have shown that it is neuroprotective for glaucoma when given with an adjuvant (Bakalash et al., 2002; Schori et al., 2001; Schwartz and Kipnis, 2002). Cop-1, also known as glatiramer acetate, immunologically cross-reacts with a wide variety of self-reactive T cells. Accordingly, its activity is reminiscent of that of altered peptide ligand, a self-peptide that has been altered and has lost pathogenicity as a result (Kipnis and Schwartz, 2002).

Cop 1 (Copolymer 1 or glatiramer acetate) is a non-pathogenic synthetic random copolymer composed of the four amino acids: L-Glu, L- Lys, L-Ala, and L-Tyr. COPAXONE®, the brand name for glatiramer acetate, consists of said four amino acids with an average molecular fraction of 0.141, 0.338, 0.427, and 0.095, respectively, and an average molecular weight of 4,700–11,000, and is currently an approved drug for the treatment of multiple sclerosis. It is very well tolerated with only minor adversary reactions. Although treatment with Cop 1 by ingestion or inhalation is disclosed in US 6,214,791, the sole route of administration of Cop 1 to multiple sclerosis patients is by daily subcutaneous injection.

WO 01/52878 and WO 01/93893 of the present applicants disclose that Cop 1, Cop 1-related peptides and polypeptides and T cells activated therewith protect CNS cells from glutamate toxicity and prevent or inhibit neuronal degeneration or promote nerve regeneration in the CNS and PNS. WO 01/93828 discloses that Cop 1 can be used for treatment of CNS disorders. None of these publications disclose the immunization with Cop 1 by administration of eye-drops containing Cop 1.

Another non-pathogenic copolymer is poly-YE, a random heterocopolymer of L-glutamic acid (Glu, E) and L-tyrosine (Tyr, Y), formerly often termed polyGT and hereinafter also called poly-Glu,Tyr, polyYE or pYE, with an average length of 100

amino acids and exhibiting a capacity to elicit strong immune response in certain mouse strains (Vidovic et al., 1985; Vidovic and Matzinger, 1988)). More than 20 years ago it was shown that several inbred as well as congenic resistant strains of mice, which fail to respond to pYE, were shown to develop specific plaque-forming cell (PFC) responses when stimulated by pYE complexed to an immunogenic carrier such as methylated bovine serum albumin (MBSA), and that pre-immunization with pEY has a tolerogenic effect on the response to YE-MBSA in some mouse strains and this tolerance can be transferred to normal, syngeneic recipients by spleen cells or thymocytes of EY-primed animals (Debre et al., 1975). None of these publications describes or suggests the use of pYE for neuroprotection.

Poly-Glu,Tyr was described in WO 03/002140 of the present applicants as active agent for preventing or inhibiting neuronal degeneration in the CNS or PNS, for promoting nerve regeneration in the CNS or PNS, or for protecting CNS or PNS cells from glutamate toxicity. This publication does not describe nor suggests the administration of poly-Glu,Tyr as eye drops for immunization.

Poultry vaccines for administration as eye drops comprising a live virus or recombinant DNA coding for immunogenic proteins from infectious agents have been described for prevention of viral diseases (Mukibi-Muka et al., 1984; Sharma, 1999; Russell and Mackie, 2001).

Citation or identification of any reference in this section or any other part of this application shall not be construed as an admission that such reference is available as prior art to the invention.

SUMMARY OF THE INVENTION

It has now been found, in accordance with the present invention, that poly-YE, when administered as an eye-drop vaccine, evokes a systemic immune response needed for neuroprotection in the CNS or PNS, as exemplified by protection of retinal ganglion cells (RGCs) against death induced by acute or chronic intraocular pressure (IOP) elevation.

The present invention thus relates, in one aspect, to an eye-drop vaccine comprising the copolymer poly-YE as active agent.

In another aspect, the present invention relates to the use of the copolymer poly-YE for the manufacture of an eye-drop vaccine.

The eye-drop vaccine according to the invention is particularly useful for therapeutic immunization of a mammal, in particular humans, for neuroprotection for treating neuronal degeneration caused by an injury, disease, disorder or condition in the central nervous system (CNS) or peripheral nervous system (PNS), for preventing or inhibiting neuronal secondary degeneration which may otherwise follow a primary injury in the CNS, for promoting nerve regeneration in the CNS or in the PNS after an injury, disease, disorder or condition, or for protecting CNS and PNS cells from glutamate toxicity.

In a further aspect, the present invention provides a method of therapeutic immunization for treating neuronal degeneration caused by an injury, disease, disorder or condition in the central nervous system (CNS) or peripheral nervous system (PNS), for preventing or inhibiting neuronal secondary degeneration which may otherwise follow a primary injury in the CNS, for promoting nerve regeneration in the CNS or in the PNS after an injury, disease, disorder or condition or for protecting CNS and PNS cells from glutamate toxicity, which comprises immunizing an individual in need with the copolymer poly-YE, in an amount effective to treat, prevent or inhibit said neuronal degeneration caused by said injury, disease, disorder or condition in the patient.

In the eye-drop vaccine of the invention, the active agent poly-YE may be administered without any adjuvant, for example in saline or phosphate-buffered saline (PBS), or it may be administered with a soluble adjuvant such as a cytokine, e.g. IL-2, IL-12, GM-CSF or IFN-γ, and the like.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows that administration of eye-drops containing poly-YE induces neuroprotection in a model of chronic glaucoma.

Fig. 2 shows that administration of eye-drops containing poly-YE induces neuroprotection in a model of acute glaucoma.

Fig. 3 shows that vaccination with eye-drops containing poly-YE has a systemic effect.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the terms "poly-YE", "polyYE", "pYE", and "poly-Glu,Tyr" are each used interchangeably to denote a random copolymer comprising residues of L-glutamic acid and L-tyrosine. Any poly-Glu,Tyr presently available or to be discovered in the future is encompassed by the present invention, but most preferred is the random copolymer poly-Glu:Tyr 1:1, most preferably the sodium salt of poly-Glu:Tyr 1:1, mol wt 20,000-50,000, preferably 20,000-40,000.

The present invention provides an eye-drop vaccine comprising as active agent the copolymer poly-YE.

In one embodiment, the eye-drop vaccine comprises the poly-YE dissolved in any suitable carrier such as saline or PBS, without any adjuvant.

In another embodiment, the eye-drop vaccine comprises the poly-YE together with a suitable soluble adjuvant such as a soluble cytokine as exemplified, but not limited to, the cytokines IL-2, IL-12, GM-CSF or IFN-γ.

The present invention further relates to a method of therapeutic immunization for neuroprotection which comprises immunizing an individual in need with an eye-drop vaccine comprising poly-YE in an amount effective to afford neuroprotection to said individual.

In one embodiment, the invention provides a method of therapeutic immunization for treating neuronal degeneration caused by an injury in the CNS or PNS, which comprises immunizing an individual in need with an eye-drop vaccine

comprising poly-YE in an amount effective to treat the neuronal degeneration caused by the injury in said individual.

In another embodiment, the invention provides a method of therapeutic immunization for preventing or inhibiting neuronal secondary degeneration which may otherwise follow a primary injury in the CNS, which comprises immunizing an individual in need with an eye-drop vaccine comprising poly-YE in an amount effective for preventing or inhibiting the neuronal degeneration which may follow a primary injury in the CNS of said individual.

In a further embodiment, the invention provides a method of therapeutic immunization for promoting nerve regeneration in the CNS or in the PNS after an injury, which comprises immunizing an individual in need with an eye-drop vaccine comprising poly-YE in an amount effective for promoting nerve regeneration in the CNS or in the PNS of said individual after the injury.

Any injury in the CNS or PNS can be treated according to the invention such as, but not limited to, spinal cord injury, blunt trauma, penetrating trauma, brain coup or contrecoup, hemorrhagic stroke, and ischemic stroke.

In yet another embodiment, the invention relates to a method of therapeutic immunization for treating neuronal degeneration caused by a disease, disorder or condition in the CNS or PNS, which comprises immunizing an individual in need with an eye-drop vaccine comprising poly-YE in an amount effective to treat the neuronal degeneration caused by the disease, disorder or condition in the CNS or PNS of said individual.

In yet a further embodiment, the invention relates to a method of therapeutic immunization for promoting nerve regeneration in the CNS or in the PNS after a disease, disorder or condition, which comprises immunizing an individual in need with an eye-drop vaccine comprising poly-YE in an amount effective to promote nerve regeneration in the CNS or in the PNS needed following a disease, disorder or condition of the CNS or PNS in said individual.

In still a further embodiment, the invention relates to a method of therapeutic immunization for protecting CNS and PNS cells from glutamate toxicity, which comprises immunizing an individual in need with an eye-drop vaccine comprising poly-YE in an amount effective to protect CNS or PNS cells in said individual from glutamate toxicity.

Among the diseases, disorders and conditions that may be treated according to the invention are, without being limited to, a senile dementia including Alzheimer's disease, a Parkinsonian syndrome including Parkinson's disease, facial nerve (Bell's) palsy, Huntington's chorea, a motor neuron disease including amyotrophic lateral sclerosis, a prion disease including Creutzfeldt-Jakob disease, Alper's disease, Batten disease, Cockayne syndrome, Lewy body disease, status epilepticus, carpal tunnel syndrome, intervertebral disc herniation, vitamin deficiency such as vitamin B deficiency, epilepsy, amnesia, anxiety, hyperalgesia, psychosis, seizures, oxidative stress, opiate tolerance and dependence, an autoimmune disease such as multiple sclerosis, a peripheral neuropathy associated with a disease such as amyloid polyneuropathy, diabetic neuropathy, uremic neuropathy, porphyric polyneuropathy, hypoglycemia, Sjogren-Larsson syndrome, acute sensory neuropathy, chronic ataxic neuropathy, biliary cirrhosis, primary amyloidosis, obstructive lung diseases, acromegaly, malabsorption syndromes, polycythemia vera, IgA and IgG gammapathies, complications of various drugs such as nitrofurantoin, metronidazole, isoniazid and toxins such as alcohol or organophosphates, Charcot-Marie-Tooth disease, ataxia telangiectasia, Friedreich's ataxia, adrenomyeloneuropathy, giant axonal neuropathy, Refsum's disease, Fabry's disease, lipoproteinemia, non-arteritic optic neuropathy, age-related macular degeneration, a retinal disorder such as retinal degeneration or a disease associated with abnormally elevated intraocular pressure such as glaucoma.

As mentioned in the Background of the Invention section, we have suggested in 1996 that perhaps in glaucoma, like in any acute insult to the nervous system or any neurodegenerative disease of the CNS, there is an ongoing process of

degeneration that affects neurons that spared the primary event, e.g. pressure elevation (Schwartz et al, 1996). This process is mediated by compounds that emerged as a result of the primary event or by deficit as a result of the primary risk factor, all of which create a hostile environment to neurons adjacent to the primary insult (Schwartz et al, 1996; Schwartz and Yoles, 2000a and 2000b). Recognition that such processes may take place in glaucoma, encouraged us to search for a therapy that, if given at any time, it may stop the progression of degeneration.

Indeed, since 1996 attempts are being made worldwide to identify mediators of toxicity to find ways that block, circumvent or eliminate these mediators, or to find ways to make the remaining neurons more tolerable to the hostile milieu crated by the ones that have already degenerated.

Our group discovered about four years ago that injured optic nerve or damaged retina deliver stress signal to the systemic immune system and recruit its help by leading to an evocation of T cell response directed against abundant antigens residing in the site of the stress. The immune response specificity is a way to bring T cells to the site of the lesion. Once activated, such T cells serve as a source of cytokines and neurotrophic factors affecting locally microglia, astrocytes and perhaps even neurons. In the course of our studies we realized that such an immune response is basically needed to help the body to cope with the injurious conditions and in disease conditions, like in trauma, need to be boosted (Moalem et al., 1999; Schori et al., 2001 and 2002; Kipnis et al., 2000).

We have discovered several ways that successfully boost the T cell-dependent immune response needed to protect damaged nerve from its hostile environment. A risk-free protection was found by the use of a compound that cross-reacts with a low affinity with a wide spectrum of self-antigens. One such compound is Copolymer 1 and another is poly-YE. The mechanism by which poly-YE affords neuroprotection is yet unknown: it may act through the T cell-dependent immune response or through a different, yet unknown, immune-mediated mechanism.

As described in our previous application WO 03/002140, poly-Glu,Tyr emulsified in adjuvant was found to be neuroprotective for RGCs against glutamate toxicity when injected to mice and for spinal cord injury when injected to rats. It was thus unexpected to discover that poly-YE will confer neuroprotection systemically when administered as eye drops.

In one preferred embodiment of the invention, the eye-drop vaccine comprising poly-YE is used to treat glaucoma, namely to arrest progression of glaucoma, a neurodegenerative disease of the optic nerve. As mentioned before, elevation of intraocular pressure (IOP) is often associated with chronic or acute glaucoma. However, very often, reduction of pressure is insufficient to stop disease progression. Elements of self-destruction are found to be associated with the disease progression. Here we show that a single vaccination with poly-YE, given in eye drops, was sufficient to rescue RGCs from IOP-induced loss in acute and chronic glaucoma rat models. Evidence is further provided that treatment with poly-YE is immune-mediated since the eye-drop effect is achieved when given contralaterally to the side with the elevated pressure in the eye.

The dosage of poly-YE to be administered will be determined by the physician according to the age of the patient and stage of the disease and may be chosen from a range of 0.1 to 1,000 mg, preferably from a range of 10-80 mg, more preferably 20-60 mg, to be administered in a periodical frequency, e.g. at least once a week, at least once a month or at least once every 2 or 3 months, or less frequently, but any other suitable dosage and interval between the immunizations is envisaged by the invention according to the condition of the patient. In case of an autoimmune disease such as multiple sclerosis, the administration may be made daily in one or more doses, preferably from one to three daily doses in a total of 0.1 to 1,000 mg, preferably within a range of 10-80 mg, more preferably 20-60 mg, or in alternate days.

The following examples illustrate certain features of the present invention but are not intended to limit the scope of the present invention.

EXAMPLES

Materials and Methods

Animals. Inbred adult male Lewis and Sprague-Dawley (SPD) rats (8 weeks; average weight 300 g) were supplied by the Animal Breeding Center at The Weizmann Institute of Science (Rehovot, Israel). The rats were maintained in a light- and temperature-controlled room and were matched for age and weight before each experiment. All animals were handled according to the regulations formulated by IACUC (International Animal Care and Use Committee).

Chronic glaucoma: induction of high intraocular pressure (IOP). Blockage of aqueous outflow causes an increase in IOP, which results in RGC death (Bakalash et al., 2002; Schori et al., 2001; Jia et al., 2000a; Levkovitch-Verbin et al., 2002). An increase in IOP was achieved in the right eyes of deeply anesthetized rats (ketamine hydrochloride 50 mg/kg, xylazine hydrochloride 0.5 mg/kg, injected intramuscularly) by blocking the aqueous outflow in that eye with 80–120 applications of blue-green Argon laser radiation from a Haag-Streit slit lamp. The laser beam, which was directed at three of the four episcleral veins and at 270 degrees of the limbal plexus, was applied with a power of 1 watt for 0.2 seconds, producing a spot size of 100 μm at the episcleral veins and 50 μm at the limbal plexus. At a second laser session one week later, the same parameters were used except that the spot size was 100 μm for all applications. This time the radiation was directed towards all four episcleral veins and 360 degrees of the limbal plexus (Schori et al., 2001).

Acute glaucoma: induction of high IOP. An increase in IOP was achieved in the right eyes of deeply anesthetized rats (ketamine hydrochloride 50 mg/kg, xylazine hydrochloride 0.5 mg/kg, injected intramuscularly) by inserting a 30-gauge needle connected to a polyethylene tube and a bag of normal saline (0.9%) infusion. The infusion bag was placed 1 meter above the rat's head, creating a closed loop circulation. High IOP was induced for exactly 1 hour after which the needle was removed from the eye and the hole was self-sealed.

Measurement of IOP. Most anesthetic agents cause a reduction in IOP (Jia et al., 2000b), thus precluding reliable measurement. To obtain accurate pressure measurements while the rat was in a tranquil state, we injected the rat intraperitoneally (i.p.) with 10 mg/ml acepromazine, a sedative drug that does not reduce IOP, and measured the pressure in both eyes 5 minutes later using a Tono-Pen XL tonometer (Automated Ophthalmics, Ellicott City, MD, USA), after applying Localin to the cornea. Because of the reported effect of anesthetic drugs on IOP measured by Tono-Pen (Jia et al., 2000b), we always measured at the same time after acepromazine injection and recorded the average of 10 values received from each eye. Measurements were performed every 2 days for 3 weeks, all at the same time of day.

Anatomical assessment of retinal damage caused by the increase in IOP. The hydrophilic neurotracer dye dextran tetramethylrhodamine (Rhodamine Dextran) (Molecular Probes, Oregon, USA) was applied 3 weeks after the first laser treatment directly into the intra-orbital portion of the optic nerve. Only axons that survive the high IOP and remain functional, and whose cell bodies are still viable, can take up the dye and demonstrate labeled RGCs. The rats were euthenized 24 hours after dye application and their retinas were excised, whole-mounted, and preserved in 4% paraformaldehyde. RGCs were counted under magnification of ×800 in a Zeiss fluorescent microscope. Four fields from each retina were counted, all with the same diameter (0.076 mm^2) and located at the same distance from the optic disc (Kipnis et al., 2001; Yoles et al., 2001). Eyes from untreated rats were used as a control. RGCs were counted by an observer who was blinded to the identity of the retinas.

Active immunization with no adjuvant. Poly-YE ($(pE^{50}Y^{50}$; Sigma, St. Louis, MO, Catalog No. P-0151) or Cop 1 (Teva Pharmaceutical Industries Ltd., Petach Tikva, Israel) were administered topically as eye drops after immersing the substance in PBS at a concentration of 10 mg/ml. Since each drop was of 50 microliter, we administered 1 drop every 5 minutes for a total of 10 drops in 50 minutes.

Example 1. Poly-YE in eye drops affords neuroprotection to RGCs in a model of chronic glaucoma

Induction of high IOP was done using Argon laser irradiation that blocks the outflow of aqueous humor out of the eye. Immediately after the second laser session (seven days after the first laser irradiation), poly-YE was immersed in an isotonic solution (PBS) in a concentration of 10 mg/ml and applied on the eye. Assuming that about 10% of the total amount would penetrate the blood vessels and that each drop was of 50 μl volume, a total of 10 drops were administered during a course of 50 minutes. Each drop remained on the cornea for 5 minutes, allowing the substance to penetrate into the conjunctival circulation so that at least 500 micrograms penetrate into the blood vessels that drain from the eye. Control eyes were applied with PBS in the same manner. Death rates of RGCs (mean±SD) after exposure to elevated IOP were calculated as percentage of normal eyes (controls). The results are shown in Fig. 1. The average RGC death rate 3 weeks after IOP elevation was 48.52%±1.68 in the control (n=4) and 27.33%±4.73 in the group treated with eye drops containing poly-YE (n=6) $p<0.0001$.

Example 2. Poly-YE in eye drops affords neuroprotection to RGCs in a model of acute glaucoma

High unilateral IOP was induced by inserting a 30-gauge needle connected to a polyethylene tube and normal saline (0.9%) infusion. The infusion bag was placed one meter above the rat's head. The rats were deeply anesthetized with ketamine and xylazine. High IOP was induced for exactly one hour, ten IOP measurements were taken with Tono-Pen (XL, Mentor®, Norwell, MA). The IOP generated damage was assessed two weeks later by counting the surviving RGCs dyed retrogradely with Rhodamine Dextran. Poly-YE or PBS were applied on the eye in the same manner that is described in Example 1. The results are shown in Fig. 2. RGC death rates were 58.58%±7.42 in the control group (n=4) and 31.5±4.73 in the group treated with eye drops containing poly-YE (n=6). $P<0.01$.

Example 3. Vaccination with poly-YE in eye drops has a systemic effect

Eye drop application of PBS, Cop 1 or poly-YE was done on the left eye and viable RGCs were labeled and counted two weeks later. The results are shown in Fig. 3. RGC count per mm^2 was 1454±221 in the control group (n=6), 1908±252 per mm^2 in the group treated with poly-YE (n=5), and 1982±357 in the group treated with Cop 1 (n=6). It can be seen that the difference between the groups treated with either Cop 1 or poly-YE, on one hand, and the control group (left column), on the other hand, was significant ($p<0.01$). No difference was noted between the groups treated with either Cop 1 or poly-YE.

To prove that the eye drops provide a route of immunization and not a way of local drug application, we inflicted acute rise in IOP in the right eye of the Lewis rats and applied poly-YE in eye drops to the contralateral side. The same effect was obtained as when given ipsilaterally.

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CLAIMS:

1. An eye-drop vaccine for therapeutic immunization comprising the copolymer poly-YE as the active agent.

2. An eye-drop vaccine according to claim 1 for treating neuronal degeneration caused by an injury, disease, disorder or condition in the central nervous system (CNS) or peripheral nervous system (PNS), for preventing or inhibiting neuronal secondary degeneration which may otherwise follow a primary injury in the CNS, for promoting nerve regeneration in the CNS or in the PNS after injury or disease, disorder or condition or for protecting CNS and PNS cells from glutamate toxicity.

3. An eye-drop vaccine according to claim 2, wherein said injury is spinal cord injury, blunt trauma, penetrating trauma, brain coup or contrecoup, hemorrhagic stroke, or ischemic stroke.

4. An eye-drop vaccine according to claim 2, wherein said disease, disorder or condition is a senile dementia including Alzheimer's disease, Parkinsonian syndrome including Parkinson's disease, facial nerve (Bell's) palsy, Huntington's chorea, a motor neuron disease including amyotrophic lateral sclerosis, a prion disease including Creutzfeldt-Jakob disease, Alper's disease, Batten disease, Cockayne syndrome, Lewy body disease, status epilepticus, carpal tunnel syndrome, intervertebral disc herniation, vitamin deficiency such as vitamin B deficiency, seizure disorders such as epilepsy, psychotic disorders such as schizophrenia and anxiety, amnesia, hyperalgesia, oxidative stress, opiate tolerance and dependence, an autoimmune disease such as multiple sclerosis, a peripheral neuropathy associated with a disease such as amyloid polyneuropathy, diabetic neuropathy, uremic neuropathy, porphyric polyneuropathy, hypoglycemia, Sjogren-Larsson syndrome, acute sensory neuropathy, chronic ataxic neuropathy, biliary cirrhosis, primary amyloidosis, obstructive lung diseases, acromegaly, malabsorption syndromes, polycythemia vera, IgA and IgG gammapathies, complications of various drugs such as nitrofurantoin, metronidazole, isoniazid and

toxins such as alcohol or organophosphates, Charcot-Marie-Tooth disease, ataxia telangiectasia, Friedreich's ataxia, adrenomyeloneuropathy, giant axonal neuropathy, Refsum's disease, Fabry's disease, lipoproteinemia, non-arteritic optic neuropathy, age-related macular degeneration, a retinal disorder such as retinal degeneration or a disease associated with abnormally elevated intraocular pressure such as glaucoma.

5. An eye-drop vaccine according to any one of claims 1 to 4, wherein said vaccine comprises poly-YE without an adjuvant.

6. An eye-drop vaccine according to any one of claims 1 to 4, wherein said vaccine comprises poly-YE with a soluble adjuvant.

7. An eye-drop vaccine according to claim 6, wherein said soluble adjuvant is a cytokine.

8. An eye-drop vaccine according to claim 7, wherein said cytokine is IL-2, IL-12, IFN-γ or GM-CSF.

9. An eye-drop vaccine according to claim 1, for administration periodically at a frequency of at least once every seven days, to at least once every month, to at least once every 2-3 months.

10. An eye-drop vaccine according to claim 1, for treatment of glaucoma.

11. Use of the copolymer poly-YE for the preparation of an eye-drop vaccine for therapeutic immunization.

12. Use according to claim 11, wherein said eye-drop vaccine is for treating neuronal degeneration caused by an injury, disease, disorder or condition in the central nervous system (CNS) or peripheral nervous system (PNS), for preventing or inhibiting neuronal secondary degeneration which may otherwise follow a primary injury in the CNS, for promoting nerve regeneration in the CNS or in the

PNS after an injury, disease, disorder or condition, or for protecting CNS and PNS cells from glutamate toxicity.

13. Use according to claim 12, wherein said injury is spinal cord injury, blunt trauma, penetrating trauma, brain coup or contrecoup, hemorrhagic stroke, or ischemic stroke.

14. Use according to claim 12, wherein said disease is a senile dementia including Alzheimer's disease, a Parkinsonian syndrome including Parkinson's disease, facial nerve (Bell's) palsy, Huntington's chorea, a motor neuron disease including amyotrophic lateral sclerosis, a prion disease including Creutzfeldt-Jakob disease, Alper's disease, Batten disease, Cockayne syndrome, Lewy body disease, status epilepticus, carpal tunnel syndrome, intervertebral disc herniation, vitamin deficiency such as vitamin B deficiency, epilepsy, amnesia, anxiety, hyperalgesia, psychosis, seizures, oxidative stress, opiate tolerance and dependence, an autoimmune disease such as multiple sclerosis, or a peripheral neuropathy associated with a disease such as amyloid polyneuropathy, diabetic neuropathy, uremic neuropathy, porphyric polyneuropathy, hypoglycemia, Sjogren-Larsson syndrome, acute sensory neuropathy, chronic ataxic neuropathy, biliary cirrhosis, primary amyloidosis, obstructive lung diseases, acromegaly, malabsorption syndromes, polycythemia vera, IgA and IgG gammapathies, complications of various drugs such as nitrofurantoin, metronidazole, isoniazid and toxins such as alcohol or organophosphates, Charcot-Marie-Tooth disease, ataxia telangiectasia, Friedreich's ataxia, adrenomyeloneuropathy, giant axonal neuropathy, Refsum's disease, Fabry's disease, lipoproteinemia, non-arteritic optic neuropathy, age-related macular degeneration, a retinal disorder such as retinal degeneration or a disease associated with abnormally elevated intraocular pressure such as glaucoma.

15. Use according to any one of claims 11 to 14, wherein said eye-drop vaccine comprises poly-YE without an adjuvant.

16. Use according to any one of claims 11 to 14, wherein said eye-drop vaccine comprises poly-YE with a soluble adjuvant.

17. Use according to claim 16, wherein said soluble adjuvant is a cytokine.

18. Use according to claim 17, wherein said cytokine is IL-2, IL-12, IFN-γ or GM-CSF.

19. Use according to claim 11, wherein said eye-drop vaccine is for administration periodically at a frequency of at least once every seven days, to at least once every month to at least once every 2-3 months.

20. Use according to claim 11, for treatment of glaucoma.

21. A method of therapeutic immunization for treating neuronal degeneration caused by an injury, disease, disorder or condition in the central nervous system (CNS) or peripheral nervous system (PNS), for preventing or inhibiting neuronal secondary degeneration which may otherwise follow a primary injury in the CNS, for promoting nerve regeneration in the CNS or in the PNS after an injury, disease, disorder or condition or for protecting CNS and PNS cells from glutamate toxicity, which comprises immunizing an individual in need with an eye-drop vaccine comprising the copolymer poly-YE, in an amount effective to treat, prevent or inhibit said neuronal degeneration caused by said injury, disease, disorder or condition in the patient.

22. A method according to claim 21, wherein said injury is selected from the group consisting of spinal cord injury, blunt trauma, penetrating trauma, brain coup or contrecoup, hemorrhagic stroke, and ischemic stroke.

23. A method according to claim 21, wherein said disease, disorder or condition is selected from the group consisting of a senile dementia including Alzheimer's disease, a Parkinsonian syndrome including Parkinson's disease, facial nerve (Bell's) palsy, Huntington's chorea, a motor neuron disease including amyotrophic

lateral sclerosis, a prion disease including Creutzfeldt-Jakob disease, Alper's disease, Batten disease, Cockayne syndrome, Lewy body disease, status epilepticus, carpal tunnel syndrome, intervertebral disc herniation, vitamin deficiency such as vitamin B deficiency, epilepsy, amnesia, anxiety, hyperalgesia, psychosis, seizures, oxidative stress, opiate tolerance and dependence, an autoimmune disease, or a peripheral neuropathy associated with a disease such as amyloid polyneuropathy, diabetic neuropathy, uremic neuropathy, porphyric polyneuropathy, hypoglycemia, Sjogren-Larsson syndrome, acute sensory neuropathy, chronic ataxic neuropathy, biliary cirrhosis, primary amyloidosis, obstructive lung diseases, acromegaly, malabsorption syndromes, polycythemia vera, IgA and IgG gammapathies, complications of various drugs such as nitrofurantoin, metronidazole, isoniazid and toxins such as alcohol or organophosphates, Charcot-Marie-Tooth disease, ataxia telangiectasia, Friedreich's ataxia, adrenomyeloneuropathy, giant axonal neuropathy, Refsum's disease, Fabry's disease, lipoproteinemia, non-arteritic optic neuropathy, age-related macular degeneration, a retinal disorder such as retinal degeneration, and a disease associated with abnormally elevated intraocular pressure such as glaucoma.

24. A method according to claim 21, which comprises immunization with the eye-drop vaccine comprising poly-YE without an adjuvant.

25. A method according to claim 21, which comprises immunization with the eye-drop vaccine comprising poly-YE with a soluble adjuvant.

26. A method according to claim 25, wherein said soluble adjuvant is a cytokine.

27. A method according to claim 26, wherein said cytokine is IL-2, IL-12, IFN-γ or GM-CSF.

28. A method according to claim 21, wherein said vaccine is administered periodically at a frequency of at least once every seven days to at least once every month to at least once every 2-3 months.

29. A method of therapeutic immunization of a glaucoma patient which comprises administering to the patient an eye-drop vaccine comprising poly-YE in an amount effective to treat glaucoma in said patient.

30. A method for reducing neuronal degeneration caused by the neurodegenerative effects of a disease, disorder or condition or for reducing secondary neuronal degeneration that follows the primary neuronal degeneration of an injury, in the central nervous system (CNS) or peripheral nervous system (PNS) of an individual in need thereof, which comprises immunizing the individual in need with an eye-drop vaccine comprising the copolymer poly-YE, in an amount effective to reduce said neuronal degeneration caused by the injury, disease, disorder or condition in said individual.

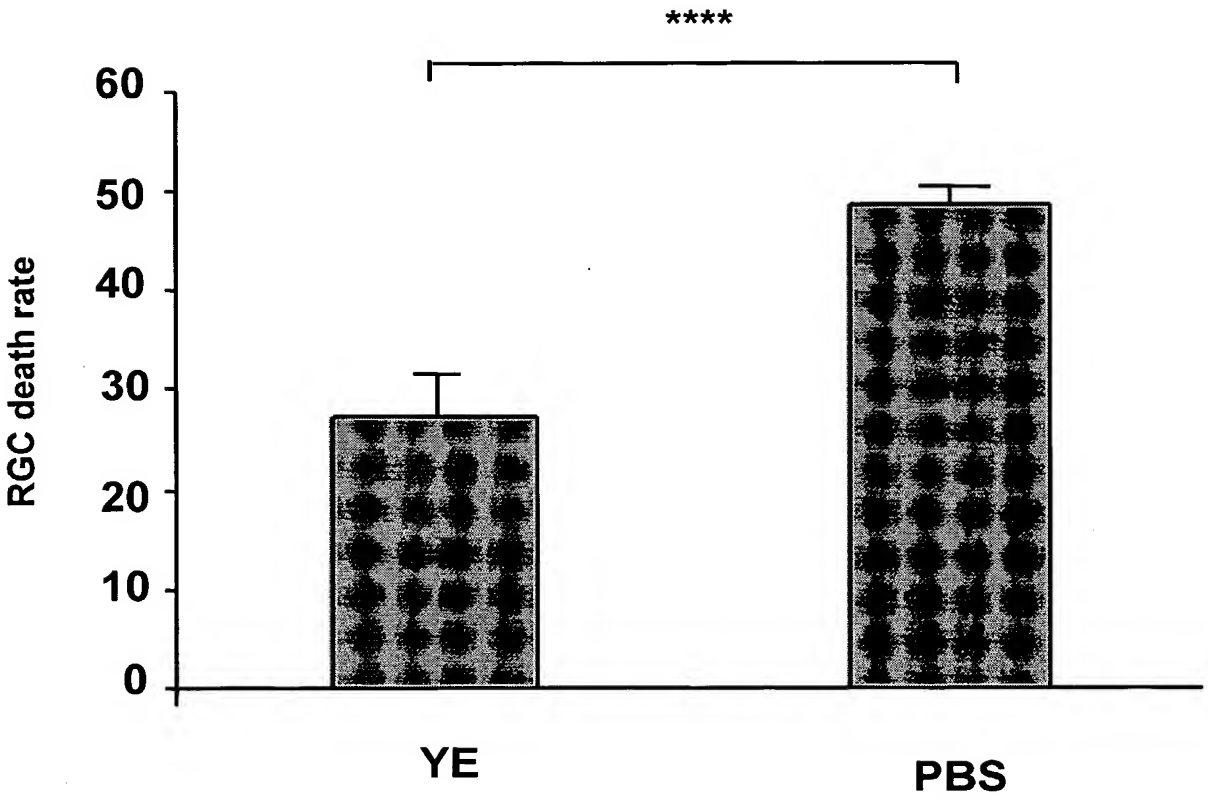

Fig. 1

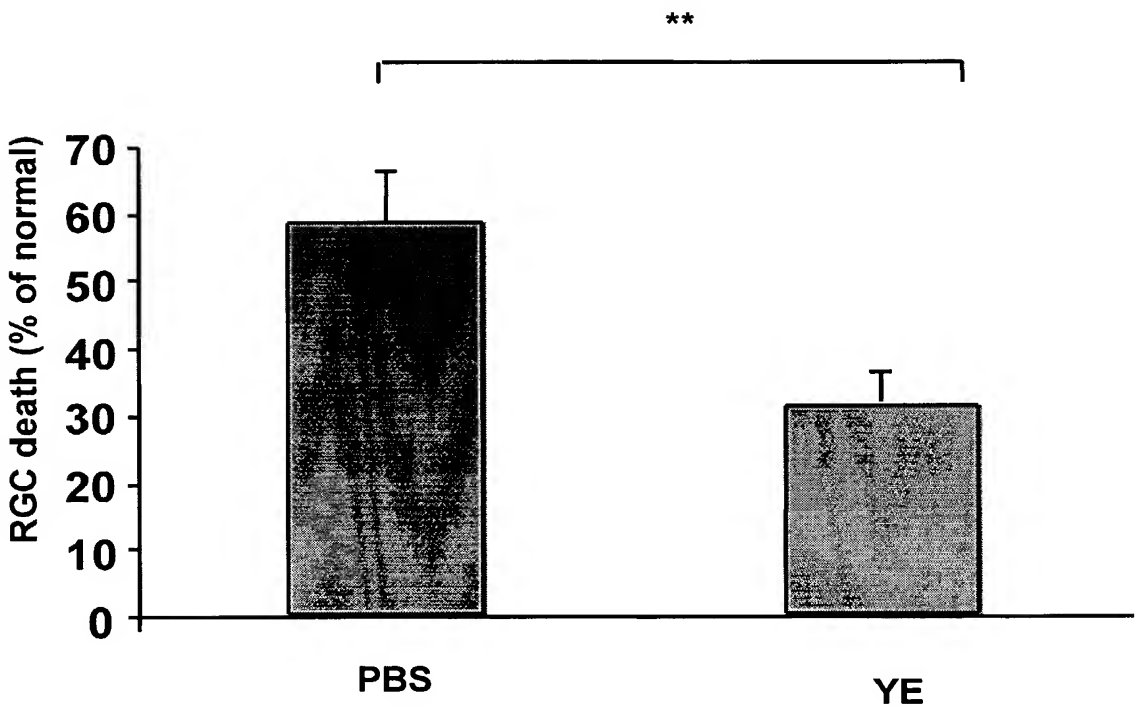

Fig. 2

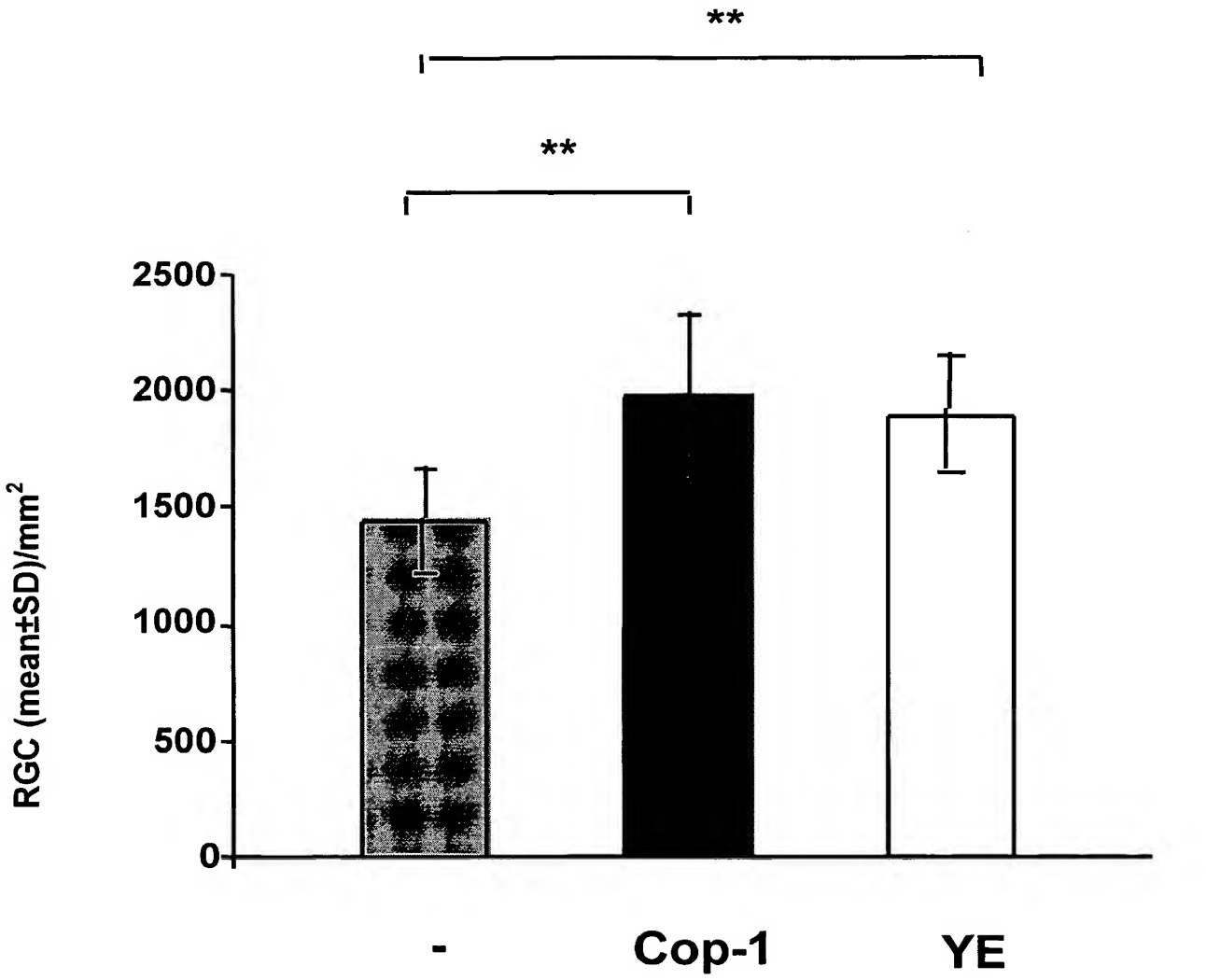

Fig. 3